

PCTA9407-2(한국생명공학연구원).TXT

&lt;110&gt; Korea Research Institute of Bioscience and Biotechnology

<120> A novel Hansenula polymorpha gene coding for alpha 1,6  
mannosyltransferase and process for the production of recombinant  
glycoproteins with Hansenula polymorpha mutant strain deficient  
in the same gene

&lt;160&gt; 14

&lt;170&gt; KopatentIn 1.71

&lt;210&gt; 1

&lt;211&gt; 1351

&lt;212&gt; DNA

&lt;213&gt; Hansenula polymorpha

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (10)..(1293)

&lt;400&gt; 1

cgg tga aga atg gtg tat ttt tta aat ttc atg tca ata acc aat gtc ccg 51  
Met Val Tyr Phe Leu Asn Phe Met Ser Ile Thr Asn Val Pro  
1 5 10

gtg ctg aag cgc gcg cga ctc tac atg gcg acg aat cgc cgg ctg gtg 99  
Val Leu Lys Arg Ala Arg Leu Tyr Met Ala Thr Asn Arg Arg Leu Val  
15 20 25 30

gtt gtt ctt gtg gtg ctg ctg tac tgg gtg gtc cag aac gtt tgg acg 147  
Val Val Leu Val Val Leu Leu Tyr Trp Val Val Gln Asn Val Trp Thr  
35 40 45

tgg agc cct ggg acg cgc gat ttg gcc caa gtg gac gcg aag atc gag 195  
Trp Ser Pro Gly Thr Arg Asp Leu Ala Gln Val Asp Ala Lys Ile Glu  
50 55 60

gcc gag cta aac tgc aat cta cat act ttt gga gcg cat ttg cgc cac 243  
Ala Glu Leu Asn Ser Asn Leu His Thr Phe Gly Ala His Leu Arg His  
65 70 75

tta aac cgg ctt ccg gca gag tgc gcc acc ctg cgt gaa aaa ctc acc 291  
Leu Asn Arg Leu Pro Ala Glu Ser Ala Thr Leu Arg Glu Lys Leu Thr  
80 85 90

ttc tat ttc cca tat tat cct gaa aag ccc gtg ccg aac cag atc tgg 339  
Phe Tyr Phe Pro Tyr Tyr Pro Glu Lys Pro Val Pro Asn Gln Ile Trp  
95 100 105 110

cag aca tgg aag gtc gat ctc gaa gac gac aac ttc ccc aag cag tac 387  
Gln Thr Trp Lys Val Asp Leu Glu Asp Asp Asn Phe Pro Lys Gln Tyr  
115 120 125

aga cgg ttt cag aag acg tgg gtc gag aaa aat cca gac tac gtg tac 435  
Arg Arg Phe Gln Lys Thr Trp Val Glu Lys Asn Pro Asp Tyr Val Tyr  
130 135 140

cac ctg att ccg gac tct gtg att gag gac ttt gtg gcg agt ttg tac 483  
His Leu Ile Pro Asp Ser Val Ile Glu Asp Phe Val Ala Ser Leu Tyr  
145 150 155

gcg aac gtg ccg gag gtg gtc aga gcg tac cag ctg ctt ccg aaa aat 531  
Ala Asn Val Pro Glu Val Val Arg Ala Tyr Gln Leu Leu Pro Lys Asn  
160 165 170

atc atg aag gcg gat ttt ttc cgg tat ttg gtg atc tac gcg cgc gga 579  
Ile Met Lys Ala Asp Phe Phe Arg Tyr Leu Val Ile Tyr Ala Arg Gly  
175 180 185 190

ggc acc tac tca gac atg gac acg gtg tgt tta aag ccg atc aag gac 627  
Gly Thr Tyr Ser Asp Met Asp Thr Val Cys Leu Lys Pro Ile Lys Asp  
195 200 205

tgg gcc acg ttt gat cgc gac ctg atc cac gct gcc gac aat aag gcc 675  
Trp Ala Thr Phe Asp Arg Asp Leu Ile His Ala Ala Asp Asn Lys Ala  
210 215 220

gat ctc tcc cag ata gat cca gaa gca aga acc acg cct gtg ggg ctg 723

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Asp Leu Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr Pro Val Gly Leu  
 225 230 235  
 gtg att ggc att gag gcc gac ccg gac agg ccc gac tgg cac gag tgg 771  
 Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp His Glu Trp  
 240 245 250  
 ttc tcg cgc aga ctg cag ttc tgc cag tgg acg atc cag gcg aag ccg 819  
 Phe Ser Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro  
 255 260 265 270  
 gga cac ccg ctg ctg cgc gag ctg atc atc cgg atc gtg gag gag acg 867  
 Gly His Pro Leu Arg Glu Leu Ile Arg Ile Val Glu Glu Thr  
 275 280 285  
 ttc cgc aaa cag cac atg ggc gtt ttg aaa aga gtg gaa ggc aag gac 915  
 Phe Arg Lys Gln His Met Gly Val Leu Lys Arg Val Glu Gly Lys Asp  
 290 295 300  
 tcg ggc gca gat atc atg cag tgg aca gga ccg ggg ata ttt aca gac 963  
 Ser Gly Ala Asp Ile Met Gln Trp Thr Gly Pro Gly Ile Phe Thr Asp  
 305 310 315  
 act ctg ttt gat tat ctg aac aat gtg cgc agc gac ggc aag ttg ggc 1011  
 Thr Leu Phe Asp Tyr Leu Asn Asn Val Ala Ser Asp Gly Lys Leu Gly  
 320 325 330  
 gac ggg tac ggc gtg ggg tgc ttg tat tgg cgc aag cac ggc aaa tat 1059  
 Asp Gly Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys His Gly Lys Tyr  
 335 340 345 350  
 aag ctg aaa aag aca gaa att aac aag aat aac gag cca ttg cat tct 1107  
 Lys Leu Lys Lys Thr Glu Ile Asn Lys Asn Asn Glu Pro Leu His Ser  
 355 360 365  
 gag gac cag ctt atc aac tgg agg tgc ctg acc aac atg gac aag cca 1155  
 Glu Asp Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn Met Asp Lys Pro  
 370 375 380  
 aag atc atg ggg gac gta atg gtg tta cca atc acg agc ttt agt ccg 1203  
 Lys Ile Met Gly Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro  
 385 390 395  
 aac gtg ggg cac atg ggc tca aag agc agc tca gat agg ctg gca ttt 1251  
 Asn Val Gly His Met Gly Ser Lys Ser Ser Ser Asp Arg Leu Ala Phe  
 400 405 410  
 gtg gag cat tta ttt tct ggc agc tgg aag cca aaa aac aaa taggaaa 1300  
 Val Glu His Leu Phe Ser Gly Ser Trp Lys Pro Lys Asn Lys  
 415 420 425  
 aataaataat tagctgcatt ttagataatt ctcatgagca ggcacagaac g 1351

<210> 2  
 <211> 428  
 <212> PRT  
 <213> Hansenula polymorpha

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 Lys Arg Ala Arg Leu Tyr Met Ala Thr Asn Arg Arg Leu Val Val Val  
 20 25 30  
 Leu Val Val Leu Leu Tyr Trp Val Val Gln Asn Val Trp Thr Trp Ser  
 35 40 45  
 Pro Gly Thr Arg Asp Leu Ala Gln Val Asp Ala Lys Ile Glu Ala Glu  
 50 55 60  
 Leu Asn Ser Asn Leu His Thr Phe Gly Ala His Leu Arg His Leu Asn  
 65 70 75 80  
 Arg Leu Pro Ala Glu Ser Ala Thr Leu Arg Glu Lys Leu Thr Phe Tyr  
 85 90 95

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Phe Pro Tyr Tyr Pro Glu Lys Pro Val Pro Asn Gln Ile Trp Gln Thr  
 100 105 110  
 Trp Lys Val Asp Leu Glu Asp Asp Asn Phe Pro Lys Gln Tyr Arg Arg  
 115 120 125  
 Phe Gln Lys Thr Trp Val Glu Lys Asn Pro Asp Tyr Val Tyr His Leu  
 130 135 140  
 Ile Pro Asp Ser Val Ile Glu Asp Phe Val Ala Ser Leu Tyr Ala Asn  
 145 150 155 160  
 Val Pro Glu Val Val Arg Ala Tyr Gln Leu Leu Pro Lys Asn Ile Met  
 165 170 175  
 Lys Ala Asp Phe Phe Arg Tyr Leu Val Ile Tyr Ala Arg Gly Gly Thr  
 180 185 190  
 Tyr Ser Asp Met Asp Thr Val Cys Leu Lys Pro Ile Lys Asp Trp Ala  
 195 200 205  
 Thr Phe Asp Arg Asp Leu Ile His Ala Ala Asp Asn Lys Ala Asp Leu  
 210 215 220  
 Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr Pro Val Gly Leu Val Ile  
 225 230 235 240  
 Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp His Glu Trp Phe Ser  
 245 250 255  
 Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His  
 260 265 270  
 Pro Leu Leu Arg Glu Leu Ile Ile Arg Ile Val Glu Glu Thr Phe Arg  
 275 280 285  
 Lys Gln His Met Gly Val Leu Lys Arg Val Glu Gly Lys Asp Ser Gly  
 290 295 300  
 Ala Asp Ile Met Gln Trp Thr Gly Pro Gly Ile Phe Thr Asp Thr Leu  
 305 310 315 320  
 Phe Asp Tyr Leu Asn Asn Val Ala Ser Asp Gly Lys Leu Gly Asp Gly  
 325 330 335  
 Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys His Gly Lys Tyr Lys Leu  
 340 345 350  
 Lys Lys Thr Glu Ile Asn Lys Asn Asn Glu Pro Leu His Ser Glu Asp  
 355 360 365  
 Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn Met Asp Lys Pro Lys Ile  
 370 375 380  
 Met Gly Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asn Val  
 385 390 395 400  
 Gly His Met Gly Ser Lys Ser Ser Ser Asp Arg Leu Ala Phe Val Glu  
 405 410 415  
 His Leu Phe Ser Gly Ser Trp Lys Pro Lys Asn Lys  
 420 425

<210> 3  
 <211> 36  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer 168Not-N

<400> 3  
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<210> 4

<211> 39  
 <212> DNA  
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<400> 4  
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39

<210> 5  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence  
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 <223> primer UNfor

<400> 5  
 ggatccccgg gtaccgagct

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<210> 6  
 <211> 20  
 <212> DNA  
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 <220>  
 <223> primer UNrev

<400> 6  
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<210> 7  
 <211> 20  
 <212> DNA  
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 <220>  
 <223> primer UCfor

<400> 7  
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<210> 8  
 <211> 20  
 <212> DNA  
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 <220>  
 <223> primer UCrev

<400> 8  
 ctggcgaaag ggggatgtgc

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<210> 9  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> primer 168Nfor

<400> 9  
 ggcggatatg gggcttcgcc

20

<210> 10

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<211> 40  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer 168Nrev

<400> 10  
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<210> 11  
 <211> 40  
 <212> DNA  
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<220>  
 <223> primer 168Cfor

<400> 11  
 gcacatcccc ctttgccag ccgatacaga gcttcagttcc 40

<210> 12  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer 168Crev

<400> 12  
 cgtcgtccgg gccagttcg 20

<210> 13  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer for the amplification of alpha1,2-mannosidase in  
 Aspergillus saitoi

<400> 13  
 ggggaattca aaaaaatggt ggtcttcagc aaa 33

<210> 14  
 <211> 69  
 <212> DNA  
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<220>  
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 Aspergillus saitoi

<400> 14  
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 caccgcgac 69